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FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		**2727-102**
		U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5) 09/508238
INTERNATIONAL APPLICATION NO. **PCT/EP98/05738**	INTERNATIONAL FILING DATE **09 September 1998 (09.09.1998)**	PRIORITY DATE CLAIMED **09 September 1997 (09.09.1997)**

TITLE OF INVENTION

"Amino Acid Sequences and Method for Isolating Bacteries from the Type Genus Pseudomonas"

APPLICANT(S) FOR DO/EO/US

Cornelia Berghof, Alexander Gasch, Anja Braeuer, Cordt Groenewald, Freimut Wilborn, Arndt Rolfs

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   - a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   - b. ☐ has been transmitted by the International Bureau.
   - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   - b. ☐ have been transmitted by the International Bureau.
   - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   - d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

Sequence Listing
WIPO Publication first page
Declaration of inventors (unsigned)

Express Mail No. EK052278656US

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5) 09/508238	INTERNATIONAL APPLICATION NO. PCT/EP98/05738	ATTORNEY'S DOCKET NUMBER 2727-102

21. The following fees are submitted:.				CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :					
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO			**$970.00**		
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO			**$840.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO			**$690.00**		
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)			**$670.00**		
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)			**$96.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =				**$840.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30				**$0.00**	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	18 - 20 =	0	x $18.00	**$0.00**	
Independent claims	3 - 3 =	0 -	x $78.00	**$0.00**	
Multiple Dependent Claims **(check if applicable).**			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$840.00**	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable).** ☐				**$0.00**	
SUBTOTAL =				**$840.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 +				**$0.00**	
TOTAL NATIONAL FEE =				**$840.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**	
TOTAL FEES ENCLOSED =				**$840.00**	
				Amount to be: refunded	$
				charged	$

☐ A check in the amount of to cover the above fees is enclosed.

☒ Please charge my Deposit Account **501145** in the amount of **$840.00** (Order No. 2727-102) to cover the above fees. A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **501145** A duplicate copy of this sheet is enclosed. (Order No. 2727-102)

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

Ronald R. Santucci
NAME

28,988
REGISTRATION NUMBER

March 9, 2000
DATE

09/508238
514 Rec'd PCT/PTO 09 MAR 2000

2727-102

IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (US/DO/EO)

Applicants: Cornelia Berghof, Alexander Gasch, Anja Braeuer, Cordt Groenewald, Freimut Wilborn, Arndt Rolfs

International Appln. No.: PCT/EP98/05738

International Filing Date: September 9, 1998

Priority Date Claimed: September 9, 1997

For: AMINO ACID SEQUENCES AND METHOD FOR ISOLATING BACTERIES FROM THE TYPE GENUS PSEUDOMONAS

PRELIMINARY AMENDMENT

Box PCT
Commissioner for Patents
Washington, D.C. 20231
Attn: DO/EO/US

S I R:

Preliminary to examination of the above-identified application kindly amend the application as follows:

In the Claims:

In claim 7, lines 1-2, kindly delete "one of the preceding claims" and substitute therefor --claim 1--;

In claim 8, lines 1-2, kindly delete "one of the preceding claims" and substitute therefor --claim 1--;

In claim 10, line 1, kindly delete "or 9";

In claim 11, lines 1-2, kindly delete "one of the preceding claims" and substitute therefor --claim 1--;

In claim 12, line 2, kindly delete "one of claims 1 to 10" and substitute therefor --claim 1--;

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Express Mail No. EK052278656US

In claim 15, line 1, kindly delete "one of claims 12 to 14" and substitute therefor --claim 12--;

In claim 17, line 1, kindly delete "one of claims 12 to 16" and substitute therefor --claim 12--.

REMARKS

The claims of the above-identified application (the claims as amended under Article 34 of the PCT) have been amended to remove all multiple dependencies. No new matter has been added. Accordingly, an early examination of the application is respectfully requested.

Respectfully submitted,

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212-687-6000

U.S 10600

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) AND 1.27 (c)) - SMALL BUSINESS CONCERN			Docket No. 2727-102
Serial No.	Filing Date	Patent No.	Issue Date
09/508,238			

Applicant/ Patentee: Cornelis Berghof, Alexander Gasch, Anja Braeuer, Cordt Groenewald, Helmut Wilborn and Arndt Rolfs

Invention: "Amino Acid Sequences and Method for Isolating Bacteries from the Type Genus Pseudomonas"

I hereby declare that I am:

- ☐ the owner of the small business concern identified below:
- ☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN: Biopesa Gesellschaft fuer Biotechnologische Entwicklung und Consulting mbH

ADDRESS OF CONCERN: Tegeler Weg 33, D-10589 Berlin, Germany

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the above identified invention described in:

- ☐ the specification filed herewith with title as listed above.
- ☒ the application identified above.
- ☐ the patent identified above.

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed on the next page and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

- ☒ no such person, concern or organization exists.
- ☐ each such person, concern or organization is listed below.

FULL NAME ____________________
ADDRESS ____________________
☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

FULL NAME ____________________
ADDRESS ____________________
☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

FULL NAME ____________________
ADDRESS ____________________
☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

FULL NAME ____________________
ADDRESS ____________________
☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: K. Berglof

TITLE OF PERSON SIGNING OTHER THAN OWNER: Director

ADDRESS OF PERSON SIGNING: Tegeler Weg 33
D-10589 Berlin
Germany

SIGNATURE: Berglof DATE: 3.03.00

09/508238
514 Rec'd PCT/PTO 09 MAR 2000

Nucleic acid sequences and processes for detecting bacteria of the *Pseudomonas* genus

The invention relates to nucleic acid molecules for detecting *Pseudomonas*, to a kit and to uses thereof.

General background of the invention

The gram-negative bacterium *Pseudomonas aeruginosa* is a widespread bacterium that is pathogenic for humans and that constitutes a major health risk especially to neonates and to people having weakened resistance. Besides its major clinical significance, the antibiotic resistances that are frequently present and the formation of toxins, especially the highly toxic exotoxin A (Woods, D.E. and Iglewski, B.H., Rev. Infect. Dis. 5, 714 - 722 (1983), *Pseudomonas aeruginosa* is one of the most important bacterial causes of cases of food poisoning. Conventional processes require at least 4 days for the detection of *Pseudomonas aeruginosa*. There is

therefore an urgent need for the development of rapid processes for detecting *Pseudomonas aeruginosa* in food and in clinical samples.

In recent years, a number of new methods have been developed for routine use in detecting particular microorganisms. These include immunological processes based on the use of polyvalent or monoclonal antibodies and processes in which nucleic acid probes are used for detection by means of hybridisation to organism-specific nucleic acids. Further methods that have been described are those processes which are based on a specific nucleic acid amplification, with or without a subsequent confirmation reaction by nucleic acid hybridisation. Processes used for the amplification of nucleic acids are, for example, the polymerase chain reaction (PCR) [US Patents 4,683,195; 4,683,202; and 4,965,188], the ligase chain reaction [WO Publication 89/09835], "self-sustained sequence replication" [EP 329,822], the "transcription based amplification system" [EP 310,229] and the Qβ RNA-replicase system [US Patent 4,957,858].

The mentioned nucleic-acid-based processes are so sensitive that, in contrast to conventional microbiological processes, it is possible to dispense with, or considerably curtail, a lengthy increase in quantity of the microorganism being detected from the sample under investigation. Testing for the presence or absence of the microorganism in question is therefore generally concluded within one day when using the mentioned nucleic-acid-based processes, thereby achieving a considerable reduction in time, especially when conventional processes require several days or weeks for detection.

Various PCR-based processes for the detection of *Pseudomonas aeruginosa* have been described. By amplifying a region of DNA having a length of 369 bp from the exotoxin A gene it has been possible to detect the presence of strains of the species *Pseudomonas aeruginosa* selectively [Khan *et al.* (1994), Appl. Environ. Microbiol. 60, 3739-3745]. Even though no bacteria of other species were detected using that PCR system, an amplified product was observed in only 96 % of the 130 *Pseudomonas aeruginosa* strains tested in total. Consequently, that PCR system is of only limited suitability for establishing a rapid process by means of which the presence of all strains of *Pseudomonas aeruginosa* can be detected reliably.

With the aid of a further, recently published process based on a multiplex PCR it has been possible to detect, selectively, fluorescent pseudomonads on the one hand and *Pseudomonas aeruginosa* on the other hand [De Vos *et al.* (1997), J. Clin. Microbiol. 35, 1295-1299]. Using that process, it was possible to detect each of the 150 isolates of *Pseudomonas aeruginosa* tested in total. It is, however, disadvantageous that the oprL gene used for the selective detection of *Pseudomonas aeruginosa* is also highly conserved in other species of the *Pseudomonas* genus. Thus, the amino acids that are coded for in the region of the binding sites of the primers used by Voss *et al.* are identical in *Pseudomonas putida* and *Pseudomonas aeruginosa*. The detection of *Pseudomonas aeruginosa* is accordingly based merely on a few different base pairs caused by the variation in the third position of particular amino acid codons, which on the basis of experience carries a high risk of false-positive and/or false-negative results occurring.

In addition, because of the high degree of conservation of the oprI and oprL genes, the multiplex PCR system described is unlikely to offer a possible means of detecting - for example by the use of various probes subsequently to the PCR reaction - other clinically significant species of the *Pseudomonas* genus, such as, for example, *Pseudomonas fluorescens*, *Pseudomonas mendocina*, *Pseudomonas putida* or *Pseudomonas stutzeri*.

An aim of the invention described herein was to establish nucleic acid sequences whose use as primers and/or probes would ensure detection, in as complete a manner as possible, of all representatives of the species *Pseudomonas aeruginosa*. A further aim of the invention was to identify a region of the genome having sufficiently high sequence variability within different species of the *Pseudomonas* genus to allow, optionally, the detection of other species of the *Pseudomonas* genus as well, for example by using different variants of primers and/or probes in the PCR or subsequently to the PCR.

Depending on the size of the group of microorganisms to be detected and the evolutionary relatedness (similarity) of microorganisms to be excluded (that are not to be detected), detection based on differential DNA sequences requires very extensive preliminary work in order to identify suitable DNA sequences that have the desired specificity in the particular case. The invention described herein relates to such DNA sequences, by means of which the rapid detection of bacteria of the *Pseudomonas* genus, especially of *Pseudomonas aeruginosa*, is possible.

Description of the invention

The problem underlying the invention is solved, according to one embodiment, by a nucleic acid molecule

that is obtainable by starting from a plurality of strains belonging to, on the one hand, a to-be-detected group of bacteria of the *Pseudomonas* genus and, on the other hand, not-to-be-detected bacteria,

(a) isolating, in a manner known *per se*, genomic DNA from a *Pseudomonas* strain of those groups (first strain),
(b) amplifying, in a manner known *per se*, the 23S/5S intergenic region, optionally together with the directly adjacent 23S region and/or the directly adjacent 5S region, and obtaining the amplification product (first amplification product),
(c) in accordance with steps (a) and (b) in each case, isolating genomic DNA using a second, third, . . . and/or n^{th} *Pseudomonas* strain of those groups, amplifying the 23S/5S intergenic region, optionally together with the directly adjacent 23S region and/or the directly adjacent 5S region, and obtaining the amplification product (second, third, . . . n^{th} amplification product),
(d) determining, in a manner known *per se*, the DNA sequence of amplification products obtained according to (b) and (c), and comparing the DNA sequence of the amplification product according to (b) with the DNA sequence of one or more amplification products according to (c), and
(e) obtaining, as a primer or probe, in a manner known *per se*, a nucleic acid molecule by means of which the to-be-detected group of bacteria of the *Pseudomonas* genus can be distinguished from the not-to-be-detected group of bacteria of the *Pseudomonas* genus on the basis of differences at at least one nucleotide position in the sequence region of the nucleic acid molecule.

The nucleic acid molecule according to the invention can be obtainable by starting from strains belonging to, on the one hand, to-be-detected bacteria of the *Pseudomonas* genus and,

on the other hand, not-to-be-detected bacteria of a genus (genera) other than *Pseudomonas*.

The problem underlying the invention is solved, according to a further embodiment, by a nucleic acid molecule that is obtainable by starting from a plurality of strains belonging to a to-be-detected group and a not-to-be-detected group of bacteria of the *Pseudomonas* genus,

(a) isolating, in a manner known *per se*, genomic DNA from a *Pseudomonas* strain of those groups (first strain),

(b) amplifying, in a manner known *per se*, the 23S/5S intergenic region, optionally together with the directly adjacent 23S region and/or the directly adjacent 5S region, and obtaining the amplification product (first amplification product),

(c) in accordance with steps (a) and (b) in each case, isolating genomic DNA using a second, third, . . . and/or n^{th} *Pseudomonas* strain of those groups, amplifying the 23S/5S intergenic region, optionally together with the directly adjacent 23S region and/or the directly adjacent 5S region, and obtaining the amplification product (second, third, . . . n^{th} amplification product),

(d) determining, in a manner known *per se*, the DNA sequence of amplification products obtained according to (b) and (c), and comparing the DNA sequence of the amplification product according to (b) with the DNA sequence of one or more amplification products according to (c), and

(e) obtaining, as a primer or probe, in a manner known *per se*, a nucleic acid molecule by means of which the to-be-detected group of bacteria of the *Pseudomonas* genus can be distinguished from the not-to-be-detected group of bacteria of the *Pseudomonas* genus on the basis of differences at at least one nucleotide position in the sequence region of the nucleic acid molecule.

The nucleic acid molecule according to the invention can be obtainable by starting from strains belonging to a to-be-detected group of bacteria of the species *Pseudomonas aeruginosa* and a not-to-be-detected group of bacteria of other species.

The invention relates also to a nucleic acid molecule of SEQ ID NO 1 or the sequence complementary thereto.

The invention relates also to a nucleic acid molecule of that kind, having a shortened sequence compared with the aforementioned nucleic acid molecule, namely the sequence of the region or in the region of the nucleotide positions 12 to 131.

The invention relates also to a nucleic acid molecule of that kind, having a shortened sequence compared with a nucleic acid molecule of SEQ ID NO 1, namely

(i) SEQ ID NO 3 or

(ii) SEQ ID NO 4 or

(iii) SEQ ID NO 5 or

(iv) the sequence complementary to each of (i), (ii) and (iii).

The invention relates also to a nucleic acid molecule of SEQ ID NO 2 or the sequence complementary thereto.

A nucleic acid molecule according to the invention may be characterised in that, in respect of its sequence in at least 10 successive nucleotides of its nucleotide chain,

(i) it is identical to a nucleic acid molecule according to one of the preceding claims or

(ii) it corresponds to a nucleic acid molecule according to one of the preceding claims in 9 out of 10 successive nucleotides or

(iii) it corresponds to a nucleic acid molecule according to one of the preceding claims in 8 out of 10 successive nucleotides or

(iv) it is at least 90 % homologous to a nucleic acid molecule according to one of the preceding claims.

Such a nucleic acid molecule according to the invention can be characterised in that it is from 10 to 250, and preferably from 15 to 30, nucleotides long.

A nucleic acid molecule according to the invention can be characterised in that it is single-stranded or double-stranded.

A nucleic acid molecule according to the invention can be characterised in that it is present

(i) as DNA or

(ii) as RNA corresponding to (i) or

(iii) as PNA,

the nucleic acid molecule where appropriate having been modified in a manner known *per se* for analytical detection processes, especially those based on hybridisation and/or amplification.

Thus, a nucleic acid molecule according to the invention can have been modified in such a manner that up to 20% of the nucleotides of at least 10 successive nucleotides of its nucleotide chain, especially 1 or 2 nucleotides, have been replaced by analogous building blocks known *per se* as probes and/or primers, especially by nucleotides that do not occur naturally in bacteria.

The nucleic acid molecule according to the invention can also have been modified or labelled or additionally modified or labelled in such a manner that it comprises, in a manner known *per se* for analytical detection processes, one or more radioactive groups, coloured groups, fluorescent groups, groups for immobilisation on a solid phase and/or groups for an indirect or direct reaction, especially for an enzymatic reaction, preferably using antibodies, antigens, enzymes and/or substances having an affinity for enzymes or enzyme complexes, and/or otherwise modifying or modified groups of nucleic-acid-like structure.

According to a further embodiment, the problem underlying the invention is solved by a kit for analytical detection processes, especially for the detection of bacteria of the *Pseudomonas* genus, that kit being characterised by one or more nucleic acid molecules according to the invention.

According to a further embodiment, the problem underlying the invention is solved by use of one or more nucleic acid molecules according to the invention or of a kit according to the invention for detection of the presence or absence of bacteria belonging to a group of bacteria of the *Pseudomonas* genus.

The use according to the invention can be characterised in that the group of bacteria of the *Pseudomonas* genus includes various strains of *Pseudomonas aeruginosa* or is made up from those strains.

Such use according to the invention can be characterised in that the group of bacteria of the *Pseudomonas* genus is composed exclusively of *Pseudomonas aeruginosa* strains.

Use according to the invention can also be characterised in that nucleic acid hybridisation and/or nucleic acid amplification is/are carried out.

Use according to the invention can also be characterised in that, as nucleic acid amplification, a polymerase chain reaction is carried out.

Use according to the invention can also be characterised in that the detection is carried out by distinguishing the to-be-detected bacteria from not-to-be-detected bacteria on the basis of differences in the genomic DNA and/or RNA at at least one nucleotide position in the region of a nucleic acid molecule according to the invention.

Use according to the invention can also be characterised in that distinguishing is carried out on the basis of differences in the region of a nucleic acid molecule of SEQ ID NO 1 or of its complementary sequence.

To detect specific microorganisms by means of nucleic acid hybridisation or amplification, organism-specific oligonucleotides are, therefore, used according to the invention. **Organism-specific oligonucleotides** are nucleic acids, from 10 to 250 bases (preferably from 15 to 30 bases) long, the base sequence of which is characteristic of a specific microorganism or a group of microorganisms. When using such organism-specific oligonucleotides (for example, as primers or probes) with the processes mentioned hereinbefore, hybridisation to DNA/amplification of DNA can take place, under suitable reaction conditions, only when the DNA of the microorganisms to be detected in the particular case is present.

Procaryotic ribosomes comprise three distinct nucleic acid components, which are generally known as 5S, 16S and 23S rRNA (ribosomal ribonucleic acid). The genetic information for those ribonucleic acids (rDNA) is arranged in the genome typically in the form of tandems. The organisation of such a unit is 16S-23S-5S, the three genes being separated from one another by short hypervariable intergenic regions. The units are present in the genome in several copies, it being possible for the number of the repeating units to vary in different bacteria. The high degree of conservation of the DNA sequence in the region of 16S rDNA, 23S rDNA and 5S rDNA across the entire kingdom of bacteria allows **non-specific oligonucleotides** to be designed, even without precise knowledge of the DNA sequences of the microorganisms to be investigated. Such non-specific oligonucleotides are characteristic of a relatively large group of microorganisms, which are generally pylogenetically related. By using those non-specific oligonucleotides it will be possible for the person skilled in the art, for example after appropriate preliminary tests by means of DNA amplification using PCR, to isolate rDNA fragments, for example the 23S/5S intergenic region, of any particular microorganism. By DNA sequencing, it is then possible to determine the sequence of the hypervariable intergenic regions of the microorganism in question.

DNA sequencing of the 23S/5S intergenic region of as large a number as possible of **to-be-detected** bacteria (e.g. of various *Pseudomonas* species), on the one hand, and subsequent comparison of those DNA sequences, on the other hand, allows DNA regions to be identified that in the group investigated (e.g. all *Pseudomonas* species) are not changed or only insignificantly changed.

DNA sequencing of the 23S/5S intergenic region of selected **not-to-be-detected** bacteria (e.g. bacteria that do not belong to the *Pseudomonas* genus), on the one hand, and subsequent comparison of those DNA sequences with the sequences of to-be-detected bacteria (e.g. various *Pseudomonas* species), on the other hand, allows DNA sequences to be identified that are characteristic of the to-be-detected bacteria (e.g. all *Pseudomonas* species). It is then possible to derive, from these DNA sequences, oligonucleotides that can be used as primers and/or probes in processes based on nucleic acids, with the aim of specifically detecting the group of bacteria in question (e.g. all species of the *Pseudomonas* genus).

The DNA sequences described in the present invention for detecting bacteria of the *Pseudomonas* genus, especially bacteria of the species *Pseudomonas aeruginosa*, are based on the 23S/5S intergenic region and the directly adjacent region of the 23S rDNA. The DNA sequence in that region was determined for a large number of bacteria. After exact sequence comparisons, organism-specific nucleic acid sequences were determined, which can be used for primers and/or probes for use in a species-/genus-specific detection process.

To detect the group of microorganisms in question, nucleic acids, preferably genomic DNA, are firstly released from the cells contained in a sample or bacterial culture to be investigated. By means of nucleic acid hybridisation, it is then possible - using the organism-specific oligonucleotides according to the invention as a probe - to **directly** detect organism-specific nucleic acids in the sample to be investigated. Various processes known to the person skilled

in the art are suitable for that purpose, such as, for example, "Southern blot" or "dot blot".

Preference is given, however, above all on account of the relatively high sensitivity, to an indirect detection process in which the DNA/RNA sequences sought are firstly amplified by means of the above-mentioned processes for amplifying nucleic acids, preferably PCR.

The amplification of DNA/RNA using the processes mentioned can be effected by using organism-specific oligonucleotides as primers, specific amplification products being formed only when DNA/RNA of the to-be-detected microorganism is present. The specificity of the detection process can be increased by a subsequent detection reaction using organism-specific oligonucleotides as probes. For that subsequent detection reaction it is also possible to use non-specific oligonucleotides.

Alternatively, the nucleic acid amplification can also be carried out in the presence of one or more non-specific oligonucleotides, so that it is possible that DNA/RNA of other, not-to-be-detected microorganisms may also be amplified. Such an amplification process is generally less specific and should therefore be backed up by a subsequent detection reaction using one or more organism-specific oligonucleotide(s) as probe(s).

Various processes by which the amplification products formed in the indirect processes can be detected will be known to the person skilled in the art. These include, *inter alia*, visualisation by means of gel electrophoresis, the hybridisation of probes on immobilised reaction products [coupled to nylon or nitrocellulose filters ("Southern

blots") or, for example, on beads or microtitre plates] and the hybridisation of the reaction products on immobilised probes (e.g. "reverse dot blots" or beads or microtitre plates coupled with probes).

A large number of different variants have been described by means of which organism-specific oligonucleotides (for example probes and primers) can be labelled or modified for the direct or indirect detection processes described. They may comprise, for example, radioactive, coloured, fluorescent or otherwise modified or modifying groups, for example antibodies, antigens, enzymes or other substances having an affinity for enzymes or enzyme complexes. Probes and primers may be either naturally occurring or synthetically produced double-stranded or single-stranded DNA or RNA or modified forms of DNA or RNA, such as, for example, PNA (in these molecules the sugar units have been replaced by amino acids or peptides). Particular nucleotides or a number of nucleotides of the probes or primers may be replaced by analogous building blocks (such as, for example, nucleotides that do not naturally occur in the target nucleic acid). In the case of the above-mentioned indirect detection processes, detection can be carried out also by means of an internally labelled amplification product. That can be effected, for example, by integrating modified nucleoside triphosphates (for example, coupled with digoxygenin or fluorescein) during the amplification reaction.

Suitable organism-specific oligonucleotides according to the invention are nucleic acids, preferably from 10 to 250 bases and especially from 15 to 30 bases long, that correspond, at least in a 10 base long sequence, to Sequences 1 to 5 mentioned hereinbelow or to their complementary sequences. Relatively small differences (1 or 2 bases) in that 10 base

long sequence are possible without the specificity mentioned in the particular case being lost in amplification and/or hybridisation. The person skilled in the art will know that in the case of such relatively small differences the reaction conditions will need to be altered accordingly; cf., for example, T. Maniatis, Molecular Cloning, Editors G. Sambrook & E.F. Fritsch, Cold Spring Harbour Laboratory Press, 1989.

The sequence of *Pseudomonas aeruginosa* (ATCC 10145) in the region of the 23S/5S intergenic region is:

(Sequence 1 = SEQ ID NO 1))
ATAACACCCAAACAATCTGAYGATTGTGTGTTGTAAGGTGAAGTCGACGAACCGAAAGTTCG
CATGAACCGCAAACACCTTGAAATCACATACCTGAATCCGGATAGACGTAAGCCCAAGCGAA
CGGATAT

In addition, the sequence in the region of the 23S/5S intergenic region was determined for 6 further strains of the species *Pseudomonas aeruginosa* and for at least one strain of each of the following species: *Pseudomonas asplenii*, *Pseudomonas citronellosis*, *Pseudomonas corrugata*, *Pseudomonas fluorescens*, *Pseudomonas fragi*, *Pseudomonas mendocina*, *Pseudomonas pseudoalcaligenes*, *Pseudomonas putida*, *Pseudomonas stutzeri*, *Pseudomonas syringae*. The sequence comparisons showed that a number of oligonucleotides derived from Sequence 1 are suitable for the selective detection of bacteria of the species *Pseudomonas aeruginosa*. The sequence of the region (12-131) is suitable for such organism-specific oligonucleotides.

From Sequence 1 there were derived the following oligonucleotides, which are especially suitable as primers for PCR (Sequence 3 and 5) and as a probe (Sequence 4).

Oligonucleotide Pa1 (Sequence 2) corresponds to position 2823-2842 of a 23S rRNA gene of *Pseudomonas aeruginosa* ATCC 10145 [Toschka *et al.* (1987), Nucleic Acids. Res. 15, 7182]:

Oligonucleotide Pa1: (Sequence 2 = SEQ ID NO 2) 5'-GATAGGCTGGGTGTGTAAGC-3'
Oligonucleotide Pa2: (Sequence 3 = SEQ ID NO 3) 5'-CTTGGGCTTACGTCTATCCG-3'
Oligonucleotide Pa3: (Sequence 4 = SEQ ID NO 4) 5'-TTCAGGTATGTGATTTCAAG GTG-3'
Oligonucleotide Pa4: (Sequence 5 = SEQ ID NO 5) 5'-GACGATTGTGTGTTGTAAGGTGA

Example 1: Detection of bacteria of the species *Pseudomonas aeruginosa* using the polymerase chain reaction

DNA was isolated by standard processes from pure cultures of the bacteria listed in Table 1. Approximately from 10 to 100 ng of each of those DNA preparations was then used in the PCR in the presence of 0.4 µM of each of oligonucleotide Pa1 and Pa2 or Pa4 and Pa2, 200 µM of dNTP's (Boehringer Mannheim), 4 mM $MgCl_2$, 16 mM $(NH_4)_2SO_4$, 67 mM Tris/HCl (pH 8.8), 0.01% Tween 20 and 0.03 U/µl Taq-polymerase (Biomaster). The PCR was carried out in a Perkin-Elmer 9600 (Pa1 and Pa2)/Biometra TRIO-Thermoblock (Pa4 and Pa2) thermocycler using the following thermoprofiles:

a) amplification using oligonucleotide Pa1 and Pa2

- initial denaturing	95°C	5 min

- 1st amplification (15 cycles)	94°C	35 sec
	68°C	30 sec
	72°C	30 sec
- 2nd amplification (20 cycles)	94°C	35 sec
	64°C	30 sec
	72°C	30 sec
- final synthesis	72°C	5 min

b) amplification using oligonucleotide Pa4 and Pa2

- initial denaturing	95°C	5 min
- amplification (35 cycles)	95°C	30 sec
	62°C	30 sec
	72°C	20 sec
- final synthesis	72°C	5 min

After the end of the PCR reaction, the amplification products were separated by means of agarose gel electrophoresis and visualised by staining with ethidium bromide. The expected products having a length of 191 bp/102 bp were observed only in those cases in which DNA of strains of the species *Pseudomonas aeruginosa* was present (compare Table 1), but not in the presence of DNA of other tested bacteria. After the end of the run, the DNA contained in the gels was transferred by standard methods to nylon filters and hybridised with the oligonucleotide Pa3 (Sequence 4) biotinylated at the 5' terminus, in order to check the specificity. Hybridisation was effected in 5 x SSC, 2 % blocking reagent, 0.1 % lauryl sarcosine, 0.02 % SDS and 5 pmol/ml of probe for 4 hours at 48°C. Washing was carried out in 2 x SSC, 0.1 % SDS for 2 x

15 minutes at room temperature and in 2 x SSC, 0.1 % SDS for 1 x 15 minutes at 48°C. Detection was carried out according to standard methods using alkaline phosphatase conjugates (Extravidin, SIGMA, # E-2636) in the presence of 5-bromo-4-chloro-3-indolyl phosphate and 4-nitro-blue tetrazolium chloride (Boehringer Mannheim).

A band was observed on the filters only in those cases in which a band had previously been visible on the agarose gel (see Table 1). Thus, the presence of all the 86 tested *Pseudomonas aeruginosa* strains was detected by PCR and by hybridisation. In contrast, none of the tested bacterial strains not belonging to that species was detected using this system.

<u>Table 1</u>: Results of PCR amplification using the oligonucleotides Pa1/Pa2 (SEQ ID NO 2 and SEQ ID NO 3) and Pa4/Pa2 (SEQ ID NO 5 and SEQ ID NO 3) and subsequent hybridisation using the oligonucleotide Pa3 (SEQ ID NO 4).

Species	**Designation of strain**	**Pa1/Pa2**	**Pa4/Pa2**
Pseudomonas aeruginosa	ATCC 9027	+	+
Pseudomonas aeruginosa	ATCC 10145	+	+
Pseudomonas aeruginosa	ATCC 14886	+	+
Pseudomonas aeruginosa	ATCC 15522	+	+
Pseudomonas aeruginosa	ATCC 15691	+	+
Pseudomonas aeruginosa	ATCC 15692	+	+
Pseudomonas aeruginosa	ATCC 21472	+	+
Pseudomonas aeruginosa	ATCC 21776	+	+
Pseudomonas aeruginosa	ATCC 33350	+	+
Pseudomonas aeruginosa	ATCC 33361	+	+
Pseudomonas aeruginosa	ATCC 33818	+	+
Pseudomonas aeruginosa	ATCC 33988	+	+
Pseudomonas aeruginosa	LMG 8029	+	+
Pseudomonas aeruginosa	DSM 288	+	+
Pseudomonas aeruginosa	DSM 939	+	+
Pseudomonas aeruginosa	DSM 1117	+	+
Pseudomonas aeruginosa	DSM 1253	+	+
Pseudomonas aeruginosa	DSM 1299	+	+
Pseudomonas aeruginosa	BC 682	+	+
Pseudomonas aeruginosa	BC 4283	+	+
Pseudomonas aeruginosa	BC 4880	+	+

Pseudomonas aeruginosa	BC 4937	+	+
Pseudomonas aeruginosa	BC 4938	+	+
Pseudomonas aeruginosa	BC 5258	+	+
Pseudomonas aeruginosa	BC 5594	+	+
Pseudomonas aeruginosa	BC 5595	+	+
Pseudomonas aeruginosa	BC 5596	+	+
Pseudomonas aeruginosa	BC 5597	+	+
Pseudomonas aeruginosa	BC 5598	+	+
Pseudomonas aeruginosa	BC 5599	+	+
Pseudomonas aeruginosa	BC 5600	+	+
Pseudomonas aeruginosa	BC 5601	+	+
Pseudomonas aeruginosa	BC 5602	+	+
Pseudomonas aeruginosa	BC 5603	+	+
Pseudomonas aeruginosa	BC 5604	+	+
Pseudomonas aeruginosa	BC 5606	+	+
Pseudomonas aeruginosa	BC 5607	+	+
Pseudomonas aeruginosa	BC 5917	+	+
Pseudomonas aeruginosa	BC 5918	+	+
Pseudomonas aeruginosa	BC 5919	+	+
Pseudomonas aeruginosa	BC 5920	+	+
Pseudomonas aeruginosa	BC 5921	+	+
Pseudomonas aeruginosa	BC 5922	+	+
Pseudomonas aeruginosa	BC 5923	+	+
Pseudomonas aeruginosa	BC 5924	+	+
Pseudomonas aeruginosa	BC 5925	+	+
Pseudomonas aeruginosa	BC 5926	+	+
Pseudomonas aeruginosa	BC 5927	+	+
Pseudomonas aeruginosa	BC 5928	+	+
Pseudomonas aeruginosa	BC 5929	+	+
Pseudomonas aeruginosa	BC 5930	+	+
Pseudomonas aeruginosa	BC 5932	+	+
Pseudomonas aeruginosa	BC 5933	+	+
Pseudomonas aeruginosa	BC 5934	+	+
Pseudomonas aeruginosa	BC 7046	+	+
Pseudomonas aeruginosa	BC 7047	+	+
Pseudomonas aeruginosa	BC 7048	+	+
Pseudomonas aeruginosa	BC 7049	+	+
Pseudomonas aeruginosa	BC 7050	+	+
Pseudomonas aeruginosa	BC 7051	+	+
Pseudomonas aeruginosa	BC 7052	+	+
Pseudomonas aeruginosa	BC 7053	+	+
Pseudomonas aeruginosa	BC 7054	+	+
Pseudomonas aeruginosa	BC 7055	+	+
Pseudomonas aeruginosa	BC 7056	+	+
Pseudomonas aeruginosa	BC 7057	+	+
Pseudomonas aeruginosa	BC 7058	+	+
Pseudomonas aeruginosa	BC 7059	+	+
Pseudomonas aeruginosa	BC 7060	+	+
Pseudomonas aeruginosa	BC 7061	+	+
Pseudomonas aeruginosa	BC 7062	+	+

Pseudomonas aeruginosa	BC 7063	+	+
Pseudomonas aeruginosa	BC 7064	+	+
Pseudomonas aeruginosa	BC 7065	+	+
Pseudomonas aeruginosa	BC 7066	+	+
Pseudomonas aeruginosa	BC 7067	+	+
Pseudomonas aeruginosa	BC 7068	+	+
Pseudomonas aeruginosa	BC 7069	+	+
Pseudomonas aeruginosa	BC 7070	+	+
Pseudomonas aeruginosa	BC 7071	+	+
Pseudomonas aeruginosa	BC 7072	+	+
Pseudomonas aeruginosa	BC 7073	+	+
Pseudomonas aeruginosa	BC 7474	n.p.	+
Pseudomonas aeruginosa	BC 7475	n.p.	+
Pseudomonas aeruginosa	BC 8468	n.p.	+
Pseudomonas aeruginosa	BC 8493	n.p.	+
Pseudomonas alcaligenes	DSM 50342	–	–
Pseudomonas asplenii	DSM 50254	–	–
Pseudomonas cepacia	BC 3134	–	–
Pseudomonas chlororaphis	BC 1753	–	–
Pseudomonas citronellosis	DSM 50332	–	–
Pseudomonas corrugata	DSM 7228	–	–
Pseudomonas fluorescens	BC 950	n.p.	–
Pseudomonas fluorescens	BC 4882	–	–
Pseudomonas fluorescens	BC 2439	–	–
Pseudomonas fragi	DSM 3456	–	–
Pseudomonas indigofera	BC 1105	n.p.	–
Pseudomonas mendocina	DSM 50017	–	[illegible]
Pseudomonas oleovorans	DSM 1045	–	–
Pseudomonas pickettii	BC 3323	–	–
Pseudomonas pseudoalcaligenes	DSM 50188	–	–
Pseudomonas putida	BC 4941	–	–
Pseudomonas putida	DSM 291	–	–
Pseudomonas putida	DSM 548	–	–
Pseudomonas putida	DSM 549	n.p.	–
Pseudomonas putida (ovalis)	ATCC 950	–	–
Pseudomonas stutzeri	BC 4940	–	–
Pseudomonas syringae	DSM 10604	–	–
Citrobacter amalonaticus	DSM 4593	–	n.p.
Enterobacter aerogenes	DSM 30053	–	n.p.
Escherichia coli	ATCC 8739	–	n.p.
Escherichia hermanii	DSM 4560	–	n.p.
Klebsiella pneumoniae	BC 5362	–	n.p.
Klebsiella terrigena	BC 4700	–	n.p.
Proteus vulgaris	DSM 2024	–	n.p.
Providencia stuartii	BC 5950	–	n.p.
Salmonella Anatum	BC 2284	–	n.p.

BC: BioteCon strain collection; n.p.: not performed.

Patent claims

1. Nucleic acid molecule obtainable by starting from a plurality of strains belonging to, on the one hand, a to-be-detected group of bacteria of the *Pseudomonas* genus and, on the other hand, not-to-be-detected bacteria,

(a) isolating, in a manner known *per se*, genomic DNA from a strain of the mentioned bacteria (first strain),

(b) amplifying, in a manner known *per se*, the 23S/5S intergenic region, optionally together with the directly adjacent 23S region and/or the directly adjacent 5S region, and obtaining the amplification product (first amplification product),

(c) in accordance with steps (a) and (b) in each case, isolating genomic DNA using a second, third, . . . and/or n^{th} strain of the mentioned bacteria, amplifying the 23S/5S intergenic region, optionally together with the directly adjacent 23S region and/or the directly adjacent 5S region, and obtaining the amplification product (second, third, . . . n^{th} amplification product),

(d) determining, in a manner known *per se*, the DNA sequence of amplification products obtained according to (b) and (c), and comparing the DNA sequence of the amplification product according to (b) with the DNA sequence of one or more amplification products according to (c), and

(e) obtaining, as a primer or probe, in a manner known *per se*, a nucleic acid molecule by means of which the to-be-detected group of bacteria of the *Pseudomonas* genus can be distinguished from not-to-be-detected bacteria, on the basis of differences at at least one nucleotide position in the sequence region of the nucleic acid molecule.

2. Nucleic acid molecule according to claim 1, obtainable by starting from strains belonging to, on the one hand, to-be-

detected bacteria of the *Pseudomonas* genus and, on the other hand, not-to-be-detected bacteria of a genus (genera) other than *Pseudomonas*.

3. Nucleic acid molecule obtainable by starting from a plurality of strains belonging to a to-be-detected group and a not-to-be-detected group of bacteria of the *Pseudomonas* genus,

(a) isolating, in a manner known *per se*, genomic DNA from a *Pseudomonas* strain of those groups (first strain),

(b) amplifying, in a manner known *per se*, the 23S/5S intergenic region, optionally together with the directly adjacent 23S region and/or the directly adjacent 5S region, and obtaining the amplification product (first amplification product),

(c) in accordance with steps (a) and (b) in each case, isolating genomic DNA using a second, third, . . . and/or n^{th} *Pseudomonas* strain of those groups, amplifying the 23S/5S intergenic region, optionally together with the directly adjacent 23S region and/or the directly adjacent 5S region, and obtaining the amplification product (second, third, . . . n^{th} amplification product),

(d) determining, in a manner known *per se*, the DNA sequence of amplification products obtained according to (b) and (c), and comparing the DNA sequence of the amplification product according to (b) with the DNA sequence of one or more amplification products according to (c), and

(e) obtaining, as a primer or probe, in a manner known *per se*, a nucleic acid molecule by means of which the to-be-detected group of bacteria of the *Pseudomonas* genus can be distinguished from the not-to-be-detected group of bacteria of the *Pseudomonas* genus on the basis of differences at at least one nucleotide position in the sequence region of the nucleic acid molecule.

4. Nucleic acid molecule according to claim 1 or 3, obtainable by starting from strains belonging to a to-be-detected group of bacteria of the species *Pseudomonas aeruginosa* and a not-to-be-detected group of bacteria of other species.

5. Nucleic acid molecule, especially according to one of the preceding claims, of SEQ ID NO 1 or the sequence complementary thereto.

6. Nucleic acid molecule having a shortened sequence compared with a nucleic acid molecule according to claim 5, namely the sequence of the region or in the region of the nucleotide positions 12 to 131.

7. Nucleic acid molecule having a shortened sequence compared with a nucleic acid molecule according to claim 5, namely
(i) SEQ ID NO 3 or
(ii) SEQ ID NO 4 or
(iii) SEQ ID NO 5 or
(iv) the sequence complementary to each of (i), (ii) and (iii).

8. Nucleic acid molecule of SEQ ID NO 2 or the sequence complementary thereto.

9. Nucleic acid molecule ***characterised*** in that, in respect of its sequence in at least 10 successive nucleotides of its nucleotide chain,
(i) it is identical to a nucleic acid molecule according to one of the preceding claims or

(ii) it corresponds to a nucleic acid molecule according to one of the preceding claims in 9 out of 10 successive nucleotides or

(iii) it corresponds to a nucleic acid molecule according to one of the preceding claims in 8 out of 10 successive nucleotides or

(iv) it is at least 90 % homologous to a nucleic acid molecule according to one of the preceding claims.

10. Nucleic acid molecule according to claim 9, ***characterised*** in that it is from 10 to 250, and preferably from 15 to 30, nucleotides long.

11. Nucleic acid molecule according to one of the preceding claims, ***characterised*** in that it is single-stranded or double-stranded.

12. Nucleic acid molecule according to one of the preceding claims, ***characterised*** in that it is present

(i) as DNA or

(ii) as RNA corresponding to (i) or

(iii) as PNA,

the nucleic acid molecule where appropriate having been modified in a manner known *per se* for analytical detection processes, especially those based on hybridisation and/or amplification.

13. Nucleic acid molecule according to claim 12, characterised in that the nucleic acid molecule has been modified in such a manner that up to 20 % of the nucleotides of at least 10 successive nucleotides of its nucleotide chain, especially 1 or 2 nucleotides, have been replaced by analogous building blocks known *per se* as probes and/or

primers, especially by nucleotides that do not occur naturally in bacteria.

14. Nucleic acid molecule according to claim 12 or 13, ***characterised*** in that the nucleic acid molecule has been modified or labelled or additionally modified or labelled in such a manner that it comprises, in a manner known *per se* for analytical detection processes, one or more radioactive groups, coloured groups, fluorescent groups, groups for immobilisation on a solid phase and/or groups for an indirect or direct reaction, especially for an enzymatic reaction, preferably using antibodies, antigens, enzymes and/or substances having an affinity for enzymes or enzyme complexes, and/or otherwise modifying or modified groups of nucleic-acid-like structure.

15. One or more nucleic acid molecules according to one of the preceding claims in the presence of optional auxiliary substances and in the form of a kit for analytical detection processes, especially for the detection of bacteria of the *Pseudomonas* genus.

16. Use of one or more nucleic acid molecules according to one of claims 1 to 14 or in the form of a kit according to claim 15 for detection of the presence or absence of bacteria belonging to a group of bacteria of the *Pseudomonas* genus.

17. Use according to claim 16, characterised in that the group of bacteria of the *Pseudomonas* genus includes various strains of *Pseudomonas aeruginosa* or is made up from those strains.

18. Use according to claim 17, characterised in that the group of bacteria of the *Pseudomonas* genus is composed exclusively of *Pseudomonas aeruginosa* strains.

19. Use according to one of claims 16 to 18, ***characterised*** in that nucleic acid hybridisation and/or nucleic acid amplification is/are carried out.

20. Use according to claim 19, ***characterised*** in that, as nucleic acid amplification, a polymerase chain reaction is carried out.

21. Use according to one of claims 16 to 20, ***characterised*** in that the detection is carried out by distinguishing the to-be-detected bacteria from not-to-be-detected bacteria on the basis of differences in the genomic DNA and/or RNA at at least one nucleotide position in the region of a nucleic acid molecule according to one of claims 1 to 14.

22. Use according to claim 21, ***characterised*** in that distinguishing is carried out on the basis of differences in the region of a nucleic acid molecule according to claim 5.

Abstract

The present invention relates to a nucleic acid molecule or molecules and to a process for the detection of bacteria of the *Pseudomonas* genus, especially *Pseudomonas aeruginosa*. The invention relates also to a test kit or kits for carrying out the said detection processes.

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

☐ Declaration Submitted with Initial Filing OR ☐ Declaration Submitted after Initial Filing

Attorney Docket Number	2727-102
First Named Inventor	Cornelia Berghof
COMPLETE IF KNOWN	
Application Number	09/508,238
Filing Date	
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

"Amino Acid Sequences and Method for Isolating Bacteries from the Type Genus Pseudomonas"

(Title of the Invention)

the specification of which

☐ is attached hereto
OR
☒ was filed on (MM/DD/YYYY) 09/09/1998 as United States Application Number or PCT International
(USSN 09/508,238)
Application Number PCT/EP98/05738 and was amended on (MM/DD/YYYY) ____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations, §1.56

I hereby claim foreign priority benefits under Title 35, United States Code §119 (a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365 (a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
197 39 611.9	Germany (DE)	09/09/1997	☐	☐	☐
			☐	☐	☐
			☐	☐	☐
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I hereby claim the benefit under Title 35, United States Code[1] 119(e) of any United States provisional application(s) listed below

Application Number(s)	Filing Date (MM/DD/YYYY)

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I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s), or §365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

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As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name: | Middle Initial: | Family Name: | Suffix e.g. Jr.:

Inventor's Signature: | Date:

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☐ Additional inventors are being named on supplemental sheet(s) attached hereto

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